Microsome preparation

Introduction

Protocol to extract the inner membranes from yeast cells. Protocol from Alex Frey.

Materials

- Lysis buffer
  - 1 x PBS
  - 1 mM DTT
  - 10 mM PMSF
  - 1 x Protease Inhibitor Cocktail (PIC), EDTA-free

Procedure

Yeast cell growth

1. Streak out the strain from a -80 °C glycerol stock on a YPD-plate and incubate at 30 °C for 2-3 days
2. Isolate a single colony and streak it out on a YPD-agar and streak it out on a YPD-agar plate and incubate 2-3 days at 30 °C
3. Inoculate a 10 ml YPD medium in a 100 ml flask with a loop of cells from a fresh plate and grow culture approximately for 18-20 h at 28 °C in a shaker (180 rpm)
4. Inoculate 2-4 x 100 ml YPD (optional: with 2.5 % DMSO, this makes the cells to form more inner membranes) at OD 0.1 in 1 l baffled flask and grow at 28 °C, 150-160 rpm for approximately 12-14 h until reaches to OD 2-2.5
5. Harvest cells by centrifugation at 8,000 rpm for 10 min at 4 °C
6. Discard supernatant and resuspend pellet with ice-cold 1x PBS, wash the pellets one more time and measure the biomass

   ALL THE FOLLOWING STEPS FOR PREPARATION OF MICROSOMES SHOULD BE CARRIED OUT AT 4 °C!

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7. Resuspend the pellet from previous step with ice-cold lysis buffer with the ratio 1:3 (w:v) and transfer it into a new tube

   From this step forward all the buffer should contain 1 mM DTT, 10 mM PMSF, PIC

8. Add equal amount of glass beads to the cell suspension and vortex at the highest speed for a min and cool it down on ice for 1 min, repeat it 10-12 times

   Check the cells under microscope for the disruption efficiency
   This step is recommended to be carried out in a cold room

9. Add fresh 10 mM PMSF after breaking of the cells is completed

   PMSF is unstable in aqueous solutions

10. Transfer broken cell suspension into a new tube, avoid transfering glass beads

11. Wash glass beads with the lysis bugger until most of the broken cells were removed from glass beads, collect all of samples and proceed to centrifugation step

12. Centrifuge at 17,000 g for 10 min to remove the cell debris and unbroken cells

13. Pour the supernatant into unltracentrifuge tubes and centrifuge for 1 h at 160,000 g at 4 °C

14. Discard the supernatant and mix microsome pellets with 1x PBS containing 5 % glycerol with the ratio of 1:10 (w:v)

    First add 1-2 ml of the buffer and resuspend it with P-1000 pipette until the pellet falls apart to small fragments, using a trimmed pipette tip will help to avoid clogging the tip

15. Transfer the microsome suspension into a pre-chilled douncer and add the rest of the buffer. Gently homogenize the mix with 10-12 strokes

16. Measure absorbance at 280